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# AN EVALUATION OF TECHNIQUES FOR SAMPLING SKIN FLORA*

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## ABSTRACT

Three methods of sampling skin bacteria were evaluated to determine whether the large differences observed from adjacent areas of the back were real or due to variability in sampling methods. It was found that in addition to actual differences in the skin flora populations of adjacent areas, there were significant differences in populations obtained by different sampling techniques and significant differences between individuals.

Equally high bacterial populations were recovered from skin by the Teflon spatula method and the rayon swab method; however, scrubbing with rayon swabs gave the most consistent results.

Among previous investigators, there has been no unanimity of opinion regarding the size of bacterial populations on the skin. Canuto (1) reported 253 organisms/cm$^2$ from the hands. Price's (2) data from the hands calculate to approximately 3200 bacteria/cm$^2$ whereas Arnold (3) found only 170 bacteria/cm$^2$ in the same location. Evans (4) noted a great variability in skin bacterial populations and attempted to minimize this by taking multiple samples within a few centimeters of each other; however, this did not completely resolve the problem. Regional differences account for some of the reported variations in skin bacterial populations. Also day to day variation in bacterial populations at the same site has been demonstrated by Pachtman (5).

Variation in the technique of securing the sample for culture appears to be partially responsible for previously reported discrepancies. One reason for such differences is that contact methods of sampling, such as plastic tape stripping (6), enumerate colonies of bacteria, while scrubbing methods tend to disperse the bacteria and enumerate individual cells. It has been demonstrated with scrubbing methods that dispersion can be increased by using detergents (such as Triton X-100) in the collecting medium (7). Ulrich has pointed out that with swabbing methods the number of bacteria recovered varies with the swabbing pressure, swabbing time, and moistening of swab head (8).

Price, however, asserts that variances in bacteria recovered from the skin reflect diversity in skin flora populations rather than variability in sampling techniques (3). He contends that the major areas of bacterial contamination are at the orifices of the sebaceous glands, and there is presumably a sparse population of bacteria distributed over the intervening skin. Variations in the distribution of sebaceous glands would, therefore, result in the differences in bacterial populations.

We have observed large differences in the number of bacteria recovered from adjacent areas of the skin of the back (9). Our studies of the bacterial skin flora among different occupational groups and the changes which occur under varying climatic conditions (10) demanded a reproducible sampling technique that could be performed easily by one person. Furthermore we wished to have a means of comparing our results with those of others.

## MATERIALS AND METHODS

*Subjects.* The backs of 10 male subjects between 14 and 60 years of age were sampled for bacteria using three different methods. Subjects were screened to insure a level of bacteria satisfactory for testing. None used antibacterial soaps, and none washed during the 24 hours preceding the sampling.

*Sampling methods.* The collection medium in all cases was normal saline phosphate buffered at pH 7.4 containing 0.1% Triton X-100. Three sampling techniques were tested: (A) Calcium algi-

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nate swabs (Colab Laboratories) moistened with collecting media were used to scrub a 16 $cm^2$ area of the back delimited by sterile plastic templates. After use, the swab head was broken off into 4 mls of collecting medium in a sterile, capped tube. All swabbing was done by the same person in a uniform manner and with an even pressure to insure consistency; (B) Moistened rayon swabs (Colab Laboratories) were used exactly as described above; (C) Teflon spatulas were used to scrape 5 $cm^2$ areas of the back delimited by sterile Teflon rings into which 2 mls of collecting medium were poured. The collecting medium was withdrawn with a syringe after one minute of agitation and was immediately added to 2 mls of collecting medium in a sterile, capped tube.

Twenty-four squares were marked on the back of each subject as shown in Figure 1. Each sampling technique was tested once on two adjacent areas on both left and right sides. The effect of applying the identical procedure twice was also tested, again using adjacent areas on both sides. For example, areas marked C1 and C1′ were duplicate sites swabbed once with calcium alginate. Areas marked C2 and C2′ received two swabbings each with alginate swabs. Areas marked R1 and R1′ were swabbed once with rayon swabs;

MAP OF SAMPLING AREA

| | | | | | |
|---|---|---|---|---|---|
| *C1 | C1¹ | ***T1 | T1 | C1¹ | C1 |
| C2 | C2¹ | T1¹ | T1¹ | C2¹ | C2 |
| **R1 | R1¹ | T2 | T2 | R1¹ | R1 |
| R2 | R2¹ | T2¹ | T2¹ | R2¹ | R2 |

LEFT RIGHT

BACK

* Calcium alginate swabs
** Rayon swabs
*** Teflon scrub

FIG. 1

areas R2 and R2′ were swabbed twice. Areas T1 and T1′ received one scrubbing with the Teflon spatula and areas T2 and T2′ received two scrubbings. The positions shown in Figure 1 were rotated in a random manner between subjects.

*Culture methods.* Each sample was mechanically shaken for ten minutes on a Burrell wrist-action

DISTRIBUTION OF BACTERIAL POPULATIONS REMOVED FROM SKIN USING ONE APPLICATION OF 3 DIFFERENT SAMPLING METHODS

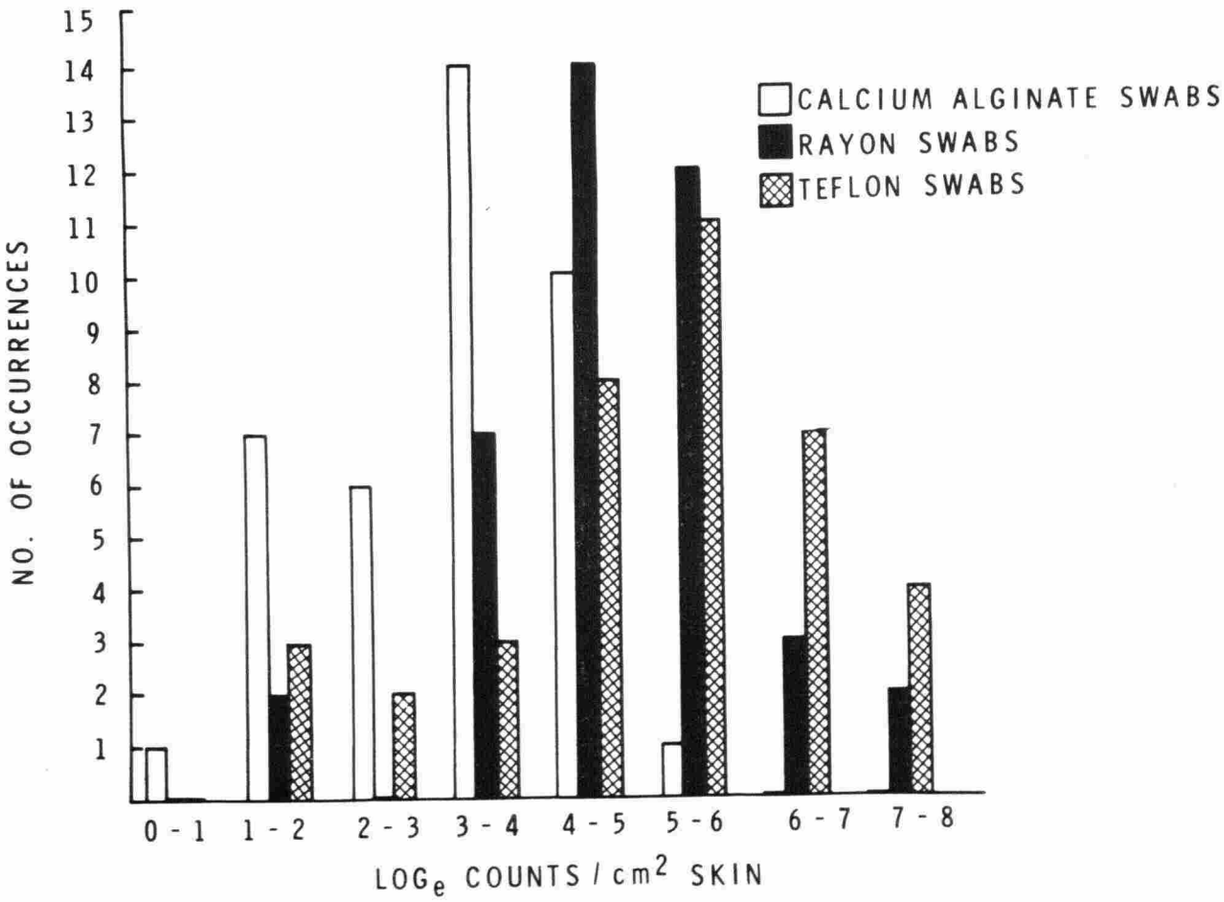


FIG. 2

shaker. One ml aliquots of each sample were used to prepare duplicate pour plates using trypticase soy agar containing 1% Tween 80. The plates were incubated aerobically for 96 hours at 31°C and were counted on a Quebec colony counter. All results were calculated in terms of number of bacteria per square centimeter of skin.

*Statistical methods.* All data were converted into logarithms ($\log_e$). Means, standard deviations, and coefficients of variation were calculated for each method for both right and left sides. An analysis of variance was used to give $P$ and $F$ values for comparison of bacteria recovered between subjects, methods, right and left sides, and one and two washings.

## RESULTS

Each sampling method received eight trials on every subject, four using one application of the technique and four trials when the same procedure was carried out twice on the same area. Thus, with ten subjects, a total of 40 values was obtained for each sampling procedure. The distribution of the 40 values representing the bacterial population recovered from skin using single washings of each of the three different methods is compared in Figure 2. The bacterial counts are grouped by their log value per sq cm of skin. The majority of counts (28 of 39, or 72%) obtained using calcium alginate swabs fell below the log 4–5 range. Rayon swabs were found to be more efficient, removing bacterial populations in the log 4–5 range or higher 78% of the time. Scrubbing with Teflon also removed bacterial populations in the log 4–5 range or higher 79% of the time.

There was a slight increase in the number of bacteria recovered from skin when each sampling procedure was performed twice on the same area. The differences between single and double washings, however, did not prove to be statistically significant in any of the three methods evaluated.

TABLE I

*Comparison of bacterial populations recovered from skin using different sampling methods*

| Sampling methods | Mean* | S.D. | Coef. of variation |
|---|---|---|---|
| Calcium alginate swabs | | | |
| Right side | 3.2 | ±1.1 | 34.82% |
| Left side | 4.1 | ±1.2 | 31.01% |
| Rayon swabs | | | |
| Right side | 4.8 | ±1.2 | 25.41% |
| Left side | 5.0 | ±1.1 | 21.12% |
| Teflon scrub | | | |
| Right side | 4.9 | ±1.6 | 32.56% |
| Left side | 4.3 | ±1.2 | 27.79% |

* $\log_e$ viable count/cm² skin.

The results are summarized in Table I where the means, standard deviations, and coefficients of variation are compared for each method. The lowest means were obtained using calcium alginate swabs. Higher means were obtained by the rayon swab method and the Teflon scrub method. When rayon swabs are used, however, the coefficient of variation is lower, indicating a greater uniformity with this method.

$F$ and $P$ values indicate that there were significant differences between subjects in the total number of bacteria recovered. On any one subject, however, the differences between total numbers of bacteria recovered from opposite sides were not statistically significant.

## DISCUSSION

As recently as 1965, Williamson stated that most earlier results on bacterial skin populations lacked reproducibility and were extremely divergent (7). The present study has shown that the large differences observed may be due to three factors: difference between bacterial populations on adjacent areas, between individuals, and in the sampling methods used. The differences in bacterial populations between individuals have been recorded (4).

A variety of factors can influence the efficacy of the different sampling methods. Ulrich reported (8) that the number of bacteria recovered from skin varies with the swabbing pressure and moistening of swab heads. In this study the rayon swab heads were attached to paper sticks considerably thicker and stronger than the wooden sticks of the calcium alginate swabs, so that more pressure could be applied without breakage. Furthermore, the rayon swabs absorb 0.27 ml of distilled water during a five minute immersion as opposed to 0.15 ml for calcium alginate. These factors could explain the differences between bacterial populations removed from skin with rayon and with calcium alginate swabs. There is no difference in the bacteriostatic properties of rayon and calcium alginate swabs (11).

Sampling done with rayon swabs yielded bacterial populations comparable in size to those

obtained with Teflon spatulas. However, the applicability of each method to various experimental situations must be kept in mind. Swabbing methods were performed with ease by one person, whereas two operators were required to carry out the Teflon scrubbing method. The additional consistency of the results obtained by the rayon swab method indicates its usefulness as an adequate sampling method where ease of sampling is a factor.

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